REMARKS

The words "and shown in Figure 1C" that appear at page 20, line 13 of the specification have been deleted to render the text consistent with the figures, specifically with Figures 1A and 1B.

Claims 1, 9, 19, 51, 58, 66, 72, 78 and 79 have been amended. Support for the amendments is found throughout the specification, for instance at page 9, line 10 through page 10, line 9, at page 16, line 20-22, in Figures 1A, 1B and 2 and in the originally filed claims. No new matter has been introduced.

Rejection of Claims 1-17, 19-24 and 51-79 Under 35 U.S.C. § 102(b)

Claims 1-17, 19-24 and 51-79 are rejected under 35 U.S.C. § 102(b) as being anticipated by Zimmerman *et al.*, *Neuron Vol. 12(1):* 11-24, (1994), as evidenced by Hogan *et al.*, *Manipulating the Mouse Embryo: A Laboratory Manual*, (Cold Spring Harbor Laboratory, 1986).

In response to Arguments presented in the Amendment filed on April 9, 2001, the Examiner stated that the issue is whether or not beta galactosidase is a "marker fluorescent protein" and that he "has established that beta galactosidase is a fluorescent protein based on the fact that it comprises numerous tryptophan residues (Office Action at page 3)." With respect to new Claims 72-77, the Examiner stated (Office Action at page 4) that these claims "do not require that the animal must be alive during the measuring steps, nor that the fluorescence of the fluorescent protein must be measured." The Examiner stated that Claim 79 "is directed to an adult transgenic mammal which has integrated into its genome DNA comprising a regulatory sequence of a mammalian nestin gene operably linked to a gene coding a fluorescent protein, wherein the fluorescent protein is expressed in multipotent stem and progenitor cells of he adult animal" and that "[t]hese characteristics are considered to be inherent in the adult mice of Zimmerman which were used to maintain the transgenic lines." (Office Action at page 4.)

Claims 1, 9, 19, 51, 58, 66 and 78 have been amended to recite that the expression of the gene coding for the (marker) fluorescent protein is detected by using fluorescence. In contrast, expression of *LacZ* beta-galactosidase is detected by histochemical staining. (See, for example,

the paragraph bridging columns 1 and 2 at page 23 of Zimmerman, *et al.*) Therefore, Claims 1-17, 19-24, 51-71 and 78 meet the requirements of 35 U.S.C. § 102(b), in view of the Zimmerman, *et al.*

As suggested by the Examiner, Claim 72 was amended to recite that fluorescence is measured from cells in a live non-human transgenic mammal, or from an organ, tissue, or region of the live non-human transgenic mammal, and that the expression of the gene coding for the fluorescent protein is detected using fluorescence. Therefore, the rejection of Claims 72-77 under 35 U.S.C. § 102(b) has been overcome.

Applicants have reviewed the text at page 14, column 1, lines 3-7 of Zimmerman, *et al.* that was discussed by the Examiner at page 4, 2nd paragraph of the Office Action. Applicants note that the Examiner has provided no evidence to support that, based on the mouse embryo and P1 data presented in the cited reference, one skilled in the art would infer that a regulatory sequence of a mammalian nestin gene, such as presently claimed by Applicants, operably linked to a gene coding for a fluorescent protein, would result in expression of the fluorescent protein in stem and progenitor cells in an adult mouse. Nevertheless, Applicants have amended Claim 79 to recite that expression of the gene coding for the fluorescent protein is detected using fluorescence. Therefore, Claim 79 also meets the requirements of 35 U.S.C. § 102(b), in view of the Zimmerman, *et al.*

Rejection of Claims 1-24, 51-71, 78 and 79 Under 35 U.S.C. § 103(a)

Claims 1-24, 51-71, 78 and 79 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Zimmerman, *et al.*, *Neuron Vol. 12(1):* 11-24, (1994), in view of Chiocchetti, *et al.*, Biochim. Biophys. Acta *Vol. 1352(2):* 193-202, (1997).

The Examiner stated that "Applicant has provided no reason why one of ordinary skill in the art, in view of the Chiochetti's disclosure that green fluorescent protein is superior to beta galactosidase, would not substitute green fluorescent protein in the construct and methods of Zimmerman."

As presently claimed, Applicants' non-human transgenic mammal, e.g., transgenic mouse, includes in its genome, DNA that comprises a regulatory sequence of a mammalian nestin gene that is operably linked to a gene coding for a (marker) fluorescent protein. The

regulatory sequence includes a nestin promoter and the second intron, or fragment thereof, of the mammalian nestin gene. The expression of the gene coding for the (marker) fluorescent protein is detected using fluorescence.

In contrast, Zimmerman, *et al.* disclose histological staining of transgenic mouse embryos and P1 newborn mouse that express *LacZ* under the control of elements of the rat nestin gene. The cited reference teaches that replacement of the rat nestin promoter with the a herpes virus thymidine kinase (TK) promoter did not affect *LacZ* expression. For instance, at page 21, 1st column, Zimmerman, *et al.* disclose that:

> To determine whether the neuroepithelial pattern of expression conferred by the second intron was the product of one or more enhancer elements capable of functioning independent of position or orientation, constructs were generated that contained only the TK promoter and nestin's second intron (upstream and downstream of the TK promoter and in both orientations) directing *lacZ* expression. Transgenic embryos expressing any of these three constructs reproduced the neuroepithelial pattern of expression charateristic of the pNesPlacZ/2ndintron plasmid (Table 1, H, I and J).

Furthermore, at page 19, column 2, lines 32-34, Zimmerman, *et al.* teach that "upstream sequences from the nestin gene are not required to drive cell type-specific gene expression."

The cited document does not teach or suggest selecting the specific elements included in Applicants' claimed regulatory sequence of a mammalian nestin gene, nor does it teach or suggest combining these specific elements with a gene coding for a (marker) fluorescent protein that is detected by fluorescence.

Chiocchetti, *et al.* teach mice in which the green fluorescent protein of jellyfish *Aequorea victoria* is placed under the control of two regulatory regions: (a) the hemopexin promoter which drives high expression of hemopexin in human adult liver and a weaker expression in some brain districts and (b) the β1-integrin distal promoter which drives ubiquitous expression during the mouse embryonic development. According to the reference, generation of transgenic mice is identified using DNA from tail biopsies. For instance, β1-GFP transgenic embryos were

identified by PCR on tail DNA, as described at page 194, paragraph bridging columns 1 and 2 of Chiochetti, *et al.*

Chiochetti, *et al.* do not remedy the deficiencies of Zimmerman, *et al.* There is no disclosure or suggestion in Chiochetti, *et al.* regarding a non-human transgenic mammal having integrated into its genome DNA that includes a regulatory sequence of a mammalian nestin gene operably linked to a gene coding for a (marker) fluorescent protein. There is no disclosure or suggestion in the cited document regarding a regulatory sequence of a mammalian nestin gene that includes a nestin promoter and the second intron or a fragment thereof, of the mammalian nestin gene, as is presently claimed by Applicants.

Thus, there is no teaching, suggestion or incentive in the cited art that supports combining their teachings (In re Geiger 2 U.S.P.Q.2d 1276, 1278 (Fed. Cir. 1987) to result in Applicants' claimed invention, particularly as amended.

Furthermore, in contrast to the teachings of the cited references, taken alone or in combination, Applicants' claimed transgenic mice exhibit surprising and unexpected properties. Specifically, their fluorescence signal is strong and sufficiently well defined to permit visual observation and identification of transgenic embryos, as discussed at page 23, lines18-21 of the subject application:

> The embryos were washed in 4°C PBS and a hand held UV light was used to determine which mice were transgenic and which were not. At this point, the entire central nervous system is expressing high levels of GFP and transgenics can easily be determined through this UV method.

See also page 25, lines 11-13 of the specification where it is stated that:

> [T]he embryos that were positive for the transgene were discerned from those that were negative by using a handheld UV lamp. The positives had a characteristic fluorescent pattern through the central nervous system.

In contrast to the surprisingly strong and well defined fluorescence pattern presented by Applicants' claimed non-human transgenic mammal, one skilled in the art would have expected a

mouse embryo, produced, as suggested by the Examiner, for example, at page 6 of the Office Action, "by substituting green fluorescent protein in the constructs and methods of Zimmerman," to display fluorescence intensity and/or resolution similar to that reported by Chiochetti, *et al.*

The Examiner's attention is directed, for example, to the description of embryos that contain the GFP gene, linked to the human β1-integrin distal promoter at page 200, column 2, lines 9-27 of the reference. In contrast to Applicants' surprising and unexpected results, Chiochetti, *et al.* identify transgenic animals by PCR analysis of tail extracted DNA. Chiochetti, *et al.* report GFP expression in two out of seven transgenic embryos. GFP expression in the two embryos is detected by fluorescence analysis, in other words, using methods and instrumentation that provide light having specific excitation wavelength and/or are capable of detecting emitted light. See, for example, Chiochetti, *et al.* at page 194, column 2 or page 195, column 1. In addition, one skilled in the art would have expected the fluorescence of a mouse embryo, produced as suggested by the Examiner, to have low signal to noise resolution, thus requiring filters, as is discussed at page 201, column 2, of Chiochetti, *et al.*, where it is stated that:

> Two different filter sets for fluorescein isothocyanate fluorescence were tested: BP 495 and BP 490-FY 455; all experiments were visualized with the latter for it gives lower background and prevents photobleaching.

The presence of unexpected results associated with Applicants' claimed invention is evidence of nonobviousness. (MPEP 716.02).

It is respectfully submitted that present Claims 1-24, 51-71, 78 and 79 are patentable over Zimmerman, *et al.*, in view of Chiocchetti, *et al.*

Rejection of Claims 51-79 Under 35 U.S.C. §103(a)

Claims 51-79 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Zimmerman, *et al.*, (*Neuron Vol. 12(1):* 11-24, 1994), in view of Chiocchetti, *et al.* (Biochim. Biophys. Acta *Vol. 1352(2):* 193-202, 1997), Yeh, *et al.* (*Proc. Nat. Acad. Sci. USA 92*:7036-7040, July, 1995), Lois, *et al.* (*Science 264*(5162):1145-1148, May,1994), and Reynolds, *et al.* (*Science 255*(5052):1707-1710, March,1992).

The Examiner stated that "Zimmerman does not teach a method of measuring multipotent stem and progenitor cells wherein the measurement step is carried out in a live animal." The Examiner also stated that "Chiochetti teaches that green fluorescent protein (GFP) is a more powerful and sensitive tool for studying gene expression in transgenic animals than is beta galactosidase." The Examiner also stated that "[i]t would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Zimmerman by substituting green fluorescent protein for beta galactosidase, and to study gene expression in neuronal stem cells in living animals and their organs and tissues." The Examiner further stated that "[o]ne would have been motivated to do so because Chiochetti teaches that GFP allows direct imaging in living cells, and suggests that changes in gene expression in living tissues could be examined through the use of GFP." The Examiner stated that "Yeh teaches that GFP can be monitored in intact, living embryos, and can be used for a variety of purposes including measurement of dynamic changes in gene expression in living tissue; lineage analysis; and monitoring cell migrations and changes in cell shape." In addition, the Examiner stated that Lois, *et al.* "teach the study of migration of neuronal precursors in adult mammalian brain," and that Reynolds "teaches that adult neuronal stem cells express nestin." The Examiner stated that "one of ordinary skill in the art would have been motivated to use a transgenic animal comprising a GFP sequence under the control of nestin regulatory sequences to follow neuoronal precursor migration in living animals."

Applicants respectfully note that the Examiner's arguments appear to address Claims 72-77, directed to a method for measuring a multipotent stem and progenitor cell population in a live animal, rather than the entire group of Claims 51-79, as indicated in the subheading for this rejection. See Office Action at page 6, line 10, through page 8, line 10. Clarification in the next Office Action is respectfully requested.

Nevertheles, Applicants respectfully submit that present Claims 51-71, 78 and 79 are patentable over Zimmerman, *et al.*, in view of Chiocchetti, *et al.* for reasons discussed above and that the additional references (Yeh, *et al.* , Lois, *et al.* and Reynolds, *et al.*,) separately or together, do not overcome the deficiencies of Zimmerman, *et al.*, in view of Chiocchetti, *et al.*

Yeh, *et al.*, disclose green fluorescent protein expression in the fly *Drosophila melanogaster*. The reference does not address non-human transgenic mammals. There is no

disclosure or suggestion in Yeh, *et al.* regarding a regulatory sequence of a mammalian nestin gene, nor regarding the combination of elements present in the regulatory sequence, claimed by Applicants. There is no disclosure or suggestion in this reference for a method for measuring a multipotent stem and progenitor cell population in a live animal or in an organ or tissue of the live animal, wherein the method includes measuring fluorescence of cells from a live non-human transgenic mammal, or organ or tissue from the live non-human transgenic mammal.

Lois, *et al.* is directed to long-distance neuronal migration in the adult mammalian brain. The reference discloses "grafted SVZ cells from adult transgenic mice that carry the reporter gene βgalactosidase attached to the promoter of the neuron-specific enolase (NSE) gene, wherein the transgene is only expressed in differentiated neurons" (Lois, *et al.* , page 1145). Lois, *et al.* do not teach or suggest the regulatory sequence of the mammalian nestin gene.

Reynolds, *et al.* is directed to the generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Reynolds, *et al.* induced cells isolated from the striatum of the adult mouse brain to proliferate in vitro by epidermal growth factor. The reference noted that the "proliferating cells initially expressed nestin" (Reynolds, *et al.*, abstract).

The combined teachings of the references cited by the Examiner do not result in Applicant's non-human transgenic mammal that exhibits a strong and well defined fluorescence to permit visual observation and identification of transgenic embryos, as discussed at page 23, lines18-21 and page 25, lines 11-13 of the subject application.

As amended, Claim 72 is directed to a method for measuring a multipotent stem and progenitor cell population in live animal, organ or tissue of the live animal, wherein the method includes measuring fluorescence of cells from a live non-human transgenic mammal, or from an organ, tissue or region of the live non-human transgenic mammal, wherein the live non-human transgenic mammal has integrated into its genome DNA comprising a regulatory sequence of a mammalian nestin gene operably linked to a gene coding for a fluorescent protein, wherein the regulatory sequence includes a nestin promoter and a second intron, or fragment thereof, of the mammalian nestin gene, and wherein the gene coding for the fluorescent protein is expressed in multipotent stem and progenitor cells of the non-human transgenic mammal and the expression of the gene coding for the fluorescent protein is detected using fluorescence, wherein the cells which fluoresce are multipotent stem and progenitor cells.

Practicing Applicants' claimed method does not require sacrificing the animal.

In contrast, to identify expression of nestin, Zimmerman, *et al.* teaches, at page 23, a whole-mount LacZ procedure and LacZ histochemistry. The data reported by Zimmerman, *et al.* is limited to embryos and P1 newborn transgenic mice. To obtain the data, the embryos or P1 newborn are sacrificed.

As described in the *Materials and Methods* section of Chiochetti, *et al.*, GFP expression in transgenic mice was studied by obtaining sections of liver or brain, or by collecting livers from adult mice. (Chiochetti, *et al.*, at page 194-195.) As with Zimmerman, *et al.*, embryos studied by fluorescence analysis also were sacrificed. (Chiochetti, *et al.* at page 200, column 2.)

As discussed above, none of the remaining references cited by the Examiner (Yeh, *et al.* Lois, *et al.* and Reynolds, *et al.*) overcome the deficiencies of Zimmerman, *et al.* and Chiochetti, *et al.* Contrary to the position taken by the Examiner in the Office Action, none of the references, taken separately or together, discloses or suggests Applicants' claimed method for measuring a multipotent stem and progenitor cell population in a live animal, organ or tissue of the live animal.

Therefore, Claims 72-77 are patentable in view of the cited references.

Information Disclosure Statement

A Third Supplemental Information Disclosure Statement (IDS) is being filed concurrently herewith. Entry of the Third Supplemental IDS is respectfully requested.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (978) 341-0036.

Respectfully submitted,

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MARKED UP VERSION OF AMENDMENTS

Specification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Please replace the paragraph at page 20, line 9 to page through page 21, line 2 with the below paragraph:

The SV40 splicing/polyadenylation region was removed from a plasmid bearing the nestin promoter (Zimmerman, L., *et al.*, *Neuron, 12*: 11-24 (1994)), poly A, and 2nd intron of the nestin gene, by cleavage with the XbaI and BamHI restriction enzymes, revealing a 250 nucleotide base pair band, and was ligated into the pBSM13+ vector (commercially available from Stratagene [and shown in Figure 1C]) which had also been cleaved by XbaI and BamHI. The XbaI site of this polyA-pBSM13+ plasmid was then blunt ended by treatment with Klenow DNA polymerase and a linker for AscI (the sequence of which is pAGGCGCGCCT) (SEQ ID. NO.: 1) was cloned into this site, reestablishing the XbaI sites on either side of the now present AscI restriction site. The second intron (1.8kb nucleotides) was digested by cutting the rat Nestin promoter/polyA/2nd intron plasmid with the restriction enzymes BamHI and SmaI, and was then ligated 3' to the poly-A-pBSM13+ plasmid which had also been cleaved using the BamHI and SmaI restriction enzymes. In order to clone the promoter sequence into the polyA/2nd intron/pBSM13+ plasmid, the HindIII site in the polyA/2nd intron/pBSM13+ plasmid was blunt ended and re-ligated, thus creating an NheI site. The nestin promoter (5.8kb nucleotides) was then digested from the rat nestin promoter/ployA/2nd intron plasmid by digesting with SpeI - SalI restriction enzymes, and was ligated to the polyA/2nd intron/pBSM13+ plasmid which had been digested with the NheI-SalI restriction enzymes, placing the nestin promoter 5' to the poly-adenylation site. The SpeI restriction site is compatible with the NheI site. In this manner, a plasmid bearing the promoter, and 2nd intron elements of the rat nestin gene with an SV40 polyadenylation sequence placed between the two was created.

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

1. (Twice Amended) A non-human transgenic mammal, progeny or embryo thereof which has integrated into its genome DNA comprising a regulatory sequence of a mammalian nestin gene operably linked to a gene coding for a marker fluorescent protein, wherein the regulatory sequence includes a nestin promoter and a second intron, or fragment thereof, of the mammalian nestin gene, and wherein the gene coding for the marker fluorescent protein is expressed in multipotent stem and progenitor cells of the non-human transgenic mammal, progeny or embryo thereof, and the expression of the gene coding for the marker fluorescent protein is detected using fluorescence.

9. (Twice Amended) A method of producing a non-human transgenic mammal which expresses a marker fluorescent protein in multipotent stem and progenitor cells, comprising:

(a) introducing into a fertilized egg of a non-human mammal, DNA comprising a regulatory sequence of a mammalian nestin gene operably linked to a gene coding for a marker fluorescent protein, wherein the regulatory sequence includes a nestin promoter and a second intron, or fragment thereof, of the mammalian nestin gene, [that] and wherein the gene coding for the marker fluorescent protein is expressed in multipotent stem and progenitor cells of the non-human mammal and expression of the gene coding for the marker fluorescent protein is detected using fluorescence;

(b) introducing the fertilized egg of (a) into a non-human mammal of the same species;

(c) allowing the non-human mammal to produce progeny which are non-human transgenic mammals; and

(d) selecting non-human mammal progeny of (c) whose multipotent stem and progenitor cells selectively express the marker fluorescent gene.

19. (Twice Amended) A method for measuring a multipotent stem and progenitor cell population in an animal organ or region thereof, comprising:

measuring cells which fluoresce from the organ or region thereof of a non-human transgenic mammal which has integrated into its genome DNA comprising:

a regulatory sequence of a mammalian nestin gene operably linked to a gene coding for a fluorescent protein, wherein the regulatory sequence includes a nestin promoter and a second intron, or fragment thereof, of the mammalian nestin gene, and wherein the gene coding for the fluorescent protein is expressed in multipotent stem and progenitor cells of the non-human transgenic mammal and the expression of the gene coding for the marker fluorescent protein is detected using fluorescence,

wherein the cells which fluoresce are multipotent stem and progenitor cells.

51. (Amended) A transgenic mouse, progeny or embryo thereof which has integrated into its genome DNA comprising a regulatory sequence of a mammalian nestin gene operably linked to a gene coding for a marker fluorescent protein, wherein the regulatory sequence includes a nestin promoter and a second intron, or fragment thereof, of the mammalian nestin gene, and wherein the gene coding for the marker fluorescent protein is expressed in multipotent stem and progenitor cells of the transgenic mouse, progeny or embryo thereof and the expression of the gene coding for the marker fluorescent protein is detected using fluorescence.

58. (Amended) A method of producing a transgenic mouse which expresses a marker fluorescent protein in multipotent stem and progenitor cells, comprising:

(a) introducing into a fertilized egg of a mouse, DNA comprising a regulatory sequence of a mammalian nestin gene operably linked to a gene coding for a marker fluorescent protein, wherein the regulatory sequence includes a nestin promoter and a second intron, or fragment thereof, of the mammalian nestin gene, and wherein the gene coding for a marker fluorescent protein [that] is expressed in multipotent stem and progenitor cells of the mouse and the expression of the gene coding for the marker fluorescent protein is detected using fluorescence;

(b) introducing the fertilized egg of (a) into a mouse;

(c) allowing the mouse to produce progeny which are transgenic mice; and

(d) selecting mice of (c) whose multipotent stem and progenitor cells selectively express the marker fluorescent gene.

66. (Amended) A method for measuring a multipotent stem and progenitor cell population in a mouse organ or region thereof, comprising:

measuring cells which fluoresce from the organ or region thereof of a transgenic mouse which has integrated into its genome DNA comprising:

a regulatory sequence of a mammalian nestin gene operably linked to a gene coding for a fluorescent protein, wherein the regulatory sequence includes a nestin promoter and a second intron, or fragment thereof, of the mammalian nestin gene, and wherein the gene coding for the fluorescent protein is expressed in multipotent stem and progenitor cells of the transgenic mouse and the expression of the gene coding for the fluorescent protein is detected using fluorescence,

wherein the cells which fluoresce are multipotent stem and progenitor cells.

72. (Amended) A method for measuring a multipotent stem and progenitor cell population in a live animal, organ or tissue [thereof] of the live animal, comprising:

measuring fluorescence of cells [which fluoresce] from [the] a live [animal] non-human transgenic mammal, or from an organ, tissue or region [thereof] of [a] the live non-human transgenic mammal, wherein [which] the live non-human transgenic mammal has integrated into its genome DNA comprising:

a regulatory sequence of a mammalian nestin gene operably linked to a gene coding for a fluorescent protein, wherein the regulatory sequence includes a nestin promoter and a second intron, or fragment thereof, of the mammalian nestin gene, and wherein the gene coding for the fluorescent protein is expressed in multipotent stem and progenitor cells of the non-human transgenic mammal and the expression of the gene coding for the fluorescent protein is detected using fluorescence,

wherein the cells which fluoresce are multipotent stem and progenitor cells.

78. (Amended) An expression construct comprising a promoter sequence of mammalian nestin gene, a gene coding for a marker fluorescent protein, wherein the marker fluorescent protein is detected using fluorescence, and a regulatory sequence present in the second intron of said mammalian nestin gene.

79. (Amended) A non-human transgenic adult mammal which has integrated into its genome DNA comprising a regulatory sequence of a mammalian nestin gene operably linked to a gene coding for a fluorescent protein, wherein the regulatory sequence includes a nestin promoter and a second intron, or fragment thereof, of the mammalian nestin gene, and wherein the gene coding for the fluorescent protein is expressed in multipotent stem and progenitor cells of the non-human transgenic adult mammal and the expression of the gene coding for the fluorescent protein is detected using fluorescence.